International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 6(4): July-August 2016

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences **Research Article……!!!**

Received: 11-06-2016; Revised: 12-07-2016; Accepted: 13-07-2016

STABILITY-INDICATING ASSAY METHOD DEVELOPMENT AND VALIDATION FOR NEBIVOLOL HYDROCHLORIDE AND CILNIDIPINE IN PHARMACEUTICAL DOSAGE FORM

Darshan C. Patel*[1], Jinal N. Tandel[2], Samir K. Shah[3]

[1]*Department of Quality Assurance, Sardar Patel College of Pharmacy, Bakrol, Gujarat.

[2]Assistant Professor, Department of Quality Assurance, Sardar Patel College of Pharmacy, Bakrol, Gujarat.

[3]Principal, Department of Pharmacology, Sardar Patel College of Pharmacy, Bakrol, Gujarat.

Keywords:

HPLC, Nebivolol Hydrochloride, Cilnidipine, Forced degradation

For Correspondence:

Darshan C. Patel

Department of Quality Assurance, Sardar Patel College of Pharmacy, Bakrol, Gujarat.

E-mail:

darshan.swami1992@gmail.com

ABSTRACT

The purpose of the present study is to develop a new, simple, rapid, accurate and precise HPLC method for simultaneous estimation of Nebivolol Hydrochloride and Cilnidipine in pharmaceutical dosage form. Stability indicating RP-HPLC method were developed and validated as per ICH guideline. In RP-HPLC, separation was achieved on Reversed-Phase C_{18} column (250 mm × 4.6 mm, 5µm) using Phosphate Buffer (pH 5.0 adjusted with 1.0 % OPA): Methanol (70:30 %v/v) as a mobile phase with flow rate 1.0 ml/min and spectrophotometric UV detection at 220 nm. Assay of marketed formulations were calculated. In RP-HPLC method, both drugs were separated by using given chromatographic condition. The NEBI and CIL were separated at 3.413 min and 6.900 min respectively. NEBI shown highest degradation in Alkaline condition 17.90% (std) and 18.22% (sample). CIL shown highest degradation in Acidic condition 19.23% (std) and 19.88% (sample). Linearity of NEBI and CIL was in the range of 2 - 10 µg/ml and 4 - 20 µg/ml, respectively. The % recoveries obtained for both drugs were 99.85-100.07 % (NEBI) and 99.89-100.12 % (CIL), respectively. The LOD values were 0.008 µg/ml and 0.0031 µg/ml, while the LOQ values were 0.024 µg/ml and 0.0096 µg/ml for NEBI and CIL respectively. Assay of formulation were found 98-102 %.

INTRODUCTION

Nebivolol Hydrochloride[I-III]

Nebivolol Hydrochloride known as (IRS, 1'RS)-I, 1'-[(2RS, 2'SR)-bis (6flurochroman- 2yl)]-2,2'-iminodiethanol hydrochloride. It is white to off white powder generally soluble in methanol. Nebivolol is a selective β1-receptor antagonist. Activation of β1-receptors by epinephrine increases the heart rate and the blood pressure and the heart consumes more oxygen. Nebivolol blocks these receptors which reverses the effects of epinephrine, lowering the heart rate and blood pressure.

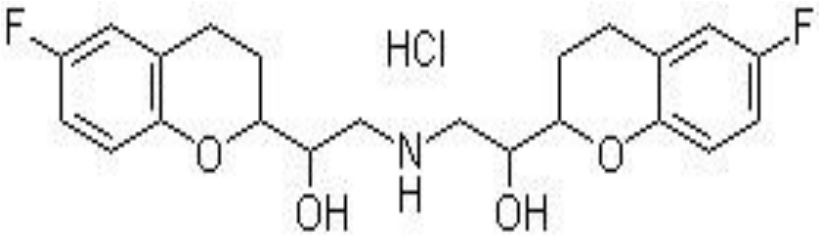

FIG I. STRUCTURE OF NEBIVOLOL HYDROCHLORIDE

Cilnidipine[IV-VI]

Cilinidipine is a dihydropyridine calcium channel blocker and chemically it is 3-O-(2Methoxyethyl) 5-O-[(E)-3-phenylprop-2-enyl] 2,6-dimethyl-4-(3-nitrophenyl)-1,4dihydropyridine-3,5-dicarboxylate and it is a unique Ca^{2+} channel blocker with an inhibitory action on the sympathetic N-type Ca^{2+} channels, which is used for patients with hypertension.

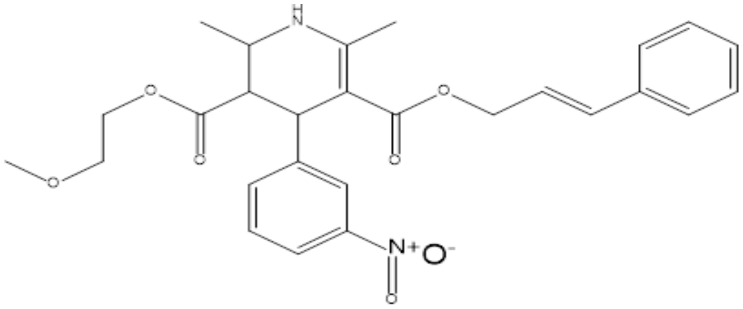

FIG II. STRUCTURE OF CILNIDIPINE

Combination of these drugs containing Nebivolol Hydrochloride (5 mg) and Cilnidpine (10 mg) are available in market as tablet. The Literature survey reveals that these drugs have been analysed individually and in combination by many analytical methods like UV[VII,VIII], HPLC[IX-XI]and Stability[XII],but there is no method available for the estimation of Nebivolol

Hydrochloride and Cilnidipine by force degradation study. Therefore, it was thought of interest to develop and validate Stability-indicating HPLC Method for the Simultaneous Estimation of Nebivolol Hydrochloride and Cilnidipine in tablet Dosage Form.

MATERIALS AND METHOD

- **Instrumentation**

Analytical Technologies HPLC System consist of S-1122 Solvent delivery system (pump), 2203 UV- Visible detector, Rheodyne injector with 20µl loop injector. Alchrome A -2000 Software system controller. Whatman filter paper no. 41, pH meter Systronics model no. 335 were used. A reverse phase C_{18} (250 mm× 4.6 mm, 5µm) analytical column was used. Weighing was done on Swisser.

- **Materials and Reagents**

Nebivolol Hydrochloride and Cilnidipine were procured as a Gift samples from Torrent Pharmaceuticals, Ahemdabad and Laksh Fine chem Anand, respectively. All Chemicals and Reagents used were of analytical or Pharmaceutical grade. HPLC grade Water, Methanol and Acetonitrile (Merck). And Ortho Phosphoric Acid, Triethyl Amine(Spectrochem)**.** The Pharmaceutical formulation used in this study was LN-βeta 5 Tablet procured from the local market and labeled to contain 5mg NEBI and 10 mg CIL per Tablet

TABLE I: OPTIMIZATION OF CHROMATOGRAPHIC CONDITION

Parameters	**Optimized condition**
Column	C_{18} (250 mm× 4.6 mm,5 µm)
Wavelength detection	220 nm
Mobile Phase composition	Phosphate Buffer (pH 5.0 adjusted with 1% Orthophosphoric acid) : Methanol (70:30 %v/v)
Column Temperature	Ambient
Injection Volume	20µl
Flow rate	1.0 ml/min

Preparation of Mobile Phase

Prepare 0.05M Potassium Dihydrogen Phosphate by dissolving 6.8 gm of Potassium Dihydrogen phosphate in 1000 ml of water. Adjust pH 5.0 with 1 % OPA solution. This solution was sonicated for 5 min for degassing and filtered through 0.45µ millipore filter. Prepare the ratio of Potassium dihydrogen Phosphate: Methanol (70:30 %v/v).

❖ **Forced degradation Study**

- **Prepare standard stock solution (API) :**

  Accurately weighed quantity of NEBI (10 mg) was transferred to 100 mL volumetric flask, dissolved and diluted up to mark with Mobile phase, to give a stock solution (100 µg/ml) and for CIL (20 mg) was transferred to 100 mL volumetric flask, dissolved and diluted up to mark with Mobile phase to give a stock (200 µg/ml).

- **Prepare stock solution (For NEBI and CIL formulation)**

  An Accurately measured tablet powder equivalent to 10 mg of NEBI and 20 mg of CIL was transferred into 100 mL volumetric flask. The content was mixed with mobile phase (10 mL), sonicated for 20 min. to dissolve the drug as completely as possible. The solution was then filtering through a Whatman filter paper no. 41. The volume was adjusted up to the mark with mobile phase.

- **Acid degradation (0.1M HCl)**

  Accurately measured 1mL of standard stock solution and 1mL of sample solution (10 µg/mL NEBI and 20 µg/mL CIL) in separately 10mL of volumetric flask. Add 2 mL of 0.1M HCl in both volumetric flask and make up volume 10mL with mobile phase. Keep this solution at 60˚c for 2 hours. After 2 hours neutralize with 0.1 M NaOH and study for acid degradation.

- **Base degradation (0.1M NaOH)**

  Accurately measured 1mL of standard stock solution and 1mL of sample solution (10 µg/mL NEBI and 20 µg/mL CIL) in separately 10mL of volumetric flask. Add 2mL of 0.1M NaOH in both volumetric flask and make up volume 10mL with mobile phase. Keep this solution at 60˚c for 2 hours. After 2 hours neutralize with 0.1 M HCl and study for base degradation.

- **Oxidation degradation (3% H_2O_2)**

  Accurately measured 1mL of standard stock solution and 1mL of sample solution (10 µg/ml NEBI and 20 µg/ml CIL) in separately 10mL of volumetric flask. Add 1mL of 3% H_2O_2 in both volumetric flask and make up volume 10mL with mobile phase. After 1 hours study for oxidation degradation.

- **Photolytic degradation (Sun light)**

  Accurately measured 1mL of standard stock solution and 1mL of sample solution (10 µg/ml NEBI and 20 µg/ml CIL) in separately 10mL of volumetric flask and make up the volume 10mL

with mobile phase. Keep the both solutions Under Sun light for 5 hours. After 5 hours study for photolytic degradation.

- **Thermal degradation (105ºC)**

Accurately measured 1mL of standard stock solution and 1mL of sample solution (10 µg/ml NEBI and 20 µg/ml CIL) in separately 10mL of volumetric flask. Make up volume 10mL with mobile phase. The solution was taken into Petri disk and stored in oven at 105ºC. After 30 min study for Thermal degradation.

❖ **Method Validation**

- As per ICH guidelines Q2R1, the method validation parameters studied were Linearity, Precision and % Recovery. [XIII]

1. Specificity

- Specificityisability tomeasurespecifically theanalyteof interestwithout any interferences from excipient and mobile phase component.For the determinationof specificity 10µg/mlsolutionofthestandardNEBI and 20µg/mlsolutionofthestandard CIL was injected. Marketed formulation of same concentration was also injected. Both chromatograms were compared and check for any interference of excipient peak. Chromatogram of blank (only mobile phase) and Placebo was also recorded to check any interference. Single standard solutions of both drugs were injected for selectivity and peak information.

2. Linearity(Calibration curve)(n=5)

- Mixed working standard solutions (0.2, 0.4, 0.6, 0.8, and 1.0 ml) equivalent to 2, 4, 6, 8, and 10 µg/ml of NEBI and 4, 8, 12, 16 and 20 µg/ml of CIL were transferred in a series of 10 ml volumetric flask and diluted to the mark with mobile phase. The solutions of each concentration were injected under the operating chromatographic conditions as described earlier. Chromatograms were recorded. Calibration curves were constructed by plotting peak areas versus concentration and the regression equations were calculated. Operations repeated five times,mean responses were calculated. %RSD was calculated. It should be less than 2%.

3. Precision

1) Intraday precision(n=3):

Take a sample of 0.2, 0.6, and 1.0 ml of working standard solution of NEBI (100 µg/ml) and CIL (200 µg/ml) were transferred to a series of 10 ml volumetric flask. The volume was

adjusted up to mark with methanol to get 2, 6, and 10 µg/ml solution of NEBI and 4, 12, and 20 µg/ml of CIL. The area of peaks were measures three different times on the same day and %RSD was calculated.

2) Interday precision(n=3):

Take a sample of 0.2, 0.6, and 1.0 ml of working standard solution of NEBI (100 µg/ml) and CIL (200 µg/ml) were transferred to a series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 2, 6, and 10 µg/ml solution of NEBI and 4, 12, and 20 µg/ml of CIL. The area of peaks were measures three different day and %RSD was calculated.

3) Repeatability(n=6):

Select the target concentration 6 µg/ml of NEBI and 12 µg/ml of CIL from linearity range. Prepare 6 solution replicate solution of target concentration in methanol. Measure the area of all replicate solution continuously at 220 nm. Calculate the standard deviation and relative standard deviation.

4) Recovery

Itwasdeterminedbycalculatingtherecovery of NEBIandCILfromformulationbystandard additionmethod.Toa fixedamountof test80%,100%and120%amountof standardwas addedandtheamountofstandardadded wascalculatedusing regressionequation.Known amountofstandardsolutionsof NEBI (3.2, 4and4.8µg/ml)andCIL(6.4, 8.0and9.6 µg/ml) wereaddedtoapre-quantifiedsample solutionof NEBIandCIL(4.0and8.0µg/ml, respectively).Eachsolutionwasinjectedintriplicateandthepercentagerecovery was calculatedbymeasuringtheresponsesandfittingthesevaluesintotheregressionequationsof the respective calibration curves.

5) Robustness

In this parameter, smallbut deliberate changes are madeto check deviationin result. This is doneto check how results remain unaffected bysmall changes that aremade.

In this, small changes aremadein to mobile phase composition, pH and flow rate.After this changes, % RSD was calculated.

6) LODandLOQ

- TheLOD(LimitofDetection)wasestimatedfromthesetof5 calibrationcurvesusedto determinemethodlinearity.TheLODmaybecalculatedas

LOD=3.3 (S.D./Slope)

Where,

SD=StandarddeviationoftheY-interceptsofthe5 calibrationcurves

Slope=Meanslopeofthe5calibrationcurves

- TheLOQ(LimitofQuantitation)wasestimatedfromthesetof5calibrationcurvesused todeterminemethodlinearity.TheLOQmaybecalculatedas

LOQ=10 (S.D./Slope)

Where,

SD=StandarddeviationoftheY-interceptsofthe5calibrationcurves

Slope=Mean slopeofthe5calibrationcurves

7) Analysis of marketed formulation

- The method was used for simultaneous estimation of NEBI and CIL in tablet dosage forms. For the sample preparation Mobile phase was used as a solvent. Ten tablets were powdered, accurately weighed (equivalent to 10 mg) and transferred in to 10 ml volumetric flask, added about 5 ml of Mobile phase in to it, sonicated for 30 minutes with intermittent shaking , cooled to attain room temperature and added up to 10 ml of Mobile phase and mixed well. It was filtered through 0.45 µ syringe filter. Further 0.1 ml of the above filtrate was diluted to 10 ml with Mobile phase to get 100 µg/ml concentration of NEBI and 200µg/ml concentration of CIL in mixture sample respectively.

RESULT

- **Final chromatogram**

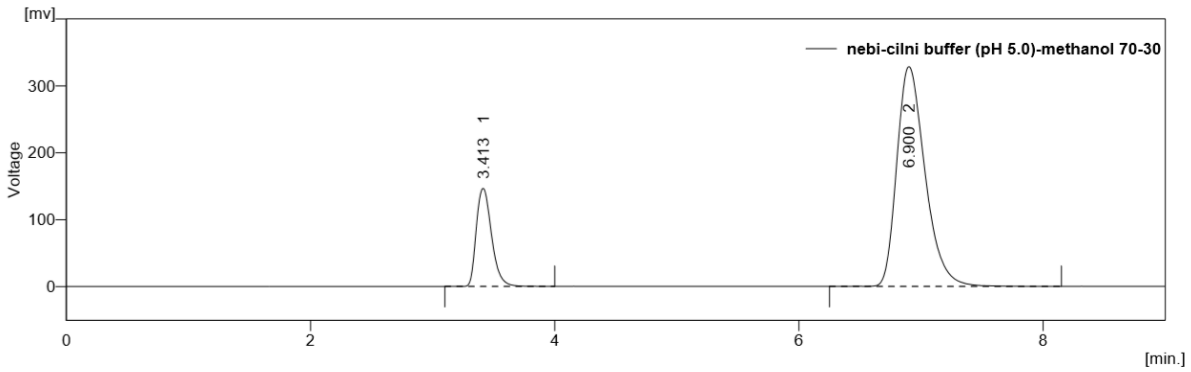

FIG. III FINAL CHROMATOGRAM OF NEBIVOLOL HYDROCHLORIDE (10µG/ML) AND CILNIDIPINE(20 µG /ML)

TABLE II. SYSTEM SUITABILITY PARAMETERS FOR FINAL CHROMATOGRAM

Name	Retention	Area	Asymmetry	Resolution	Theoretical plates
NEBI	3.413	1275.761	1.564	0.00	3456
CIL	6.900	5277.826	1.467	10.612	4220

- **Force Degradation**

1. Acid Degradation

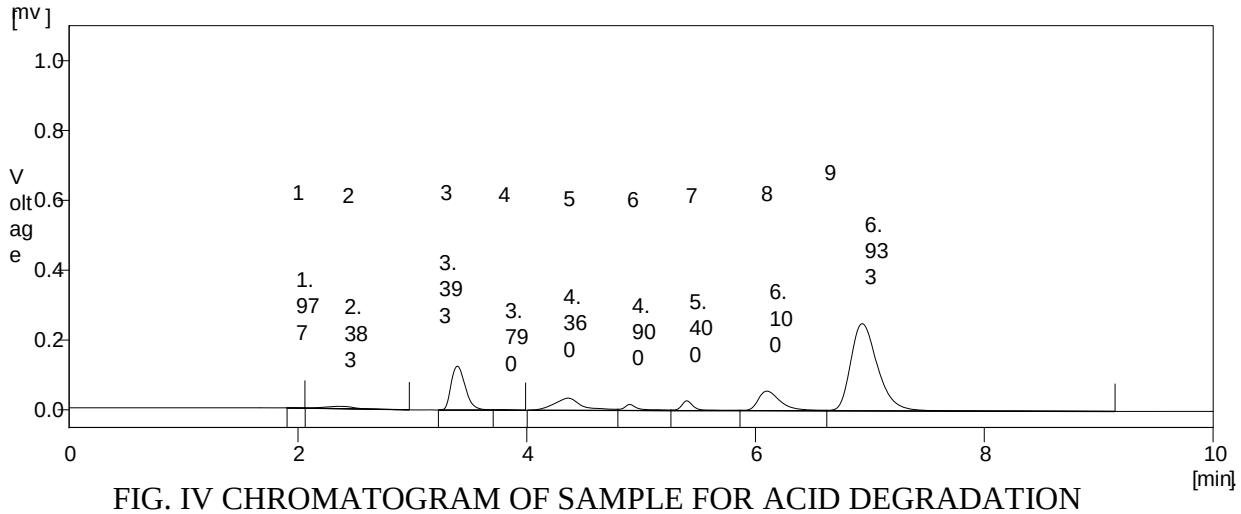

FIG. IV CHROMATOGRAM OF SAMPLE FOR ACID DEGRADATION

2. Base Degradation

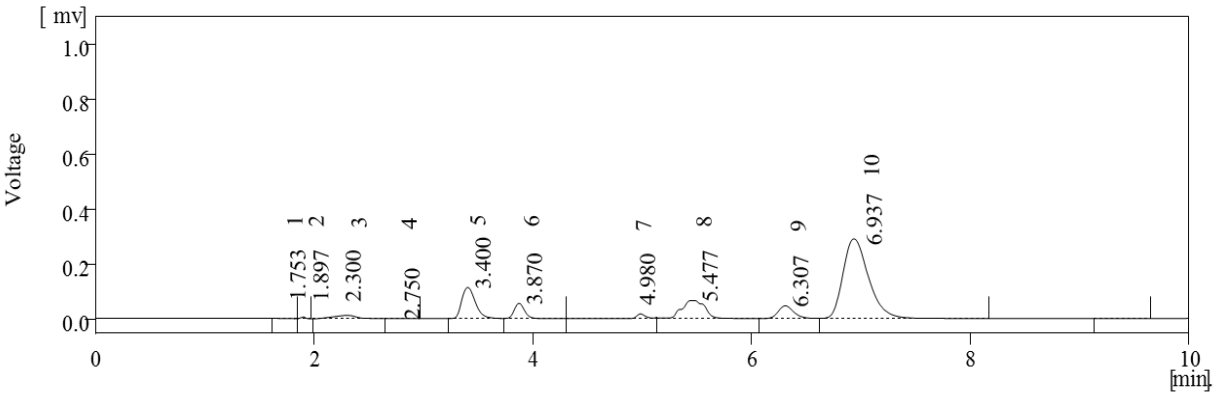

FIG. V CHROMATOGRAM OF SAMPLE FOR BASE DEGRADATION

3. Peroxide degradation

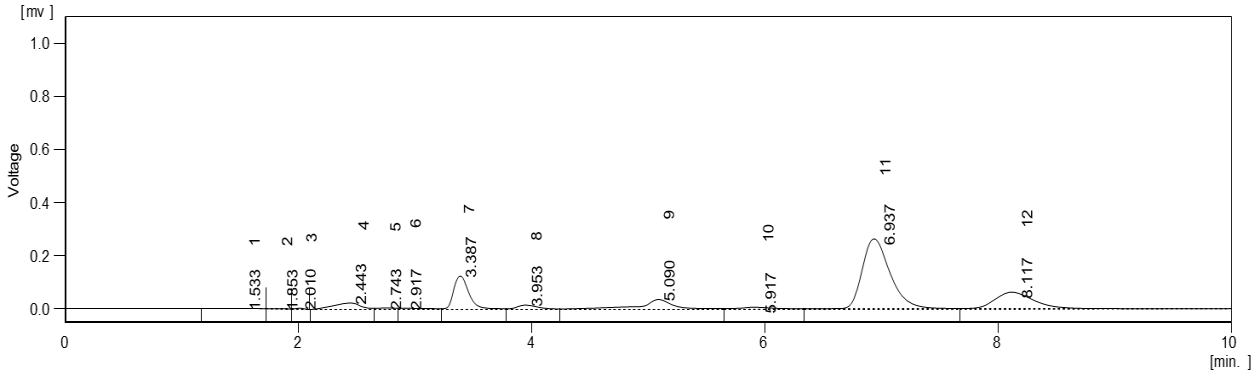

FIG. VI CHROMATOGRAM OF SAMPLE FOR PEROXIDE DEGRADATION

4. Photolytic degradation

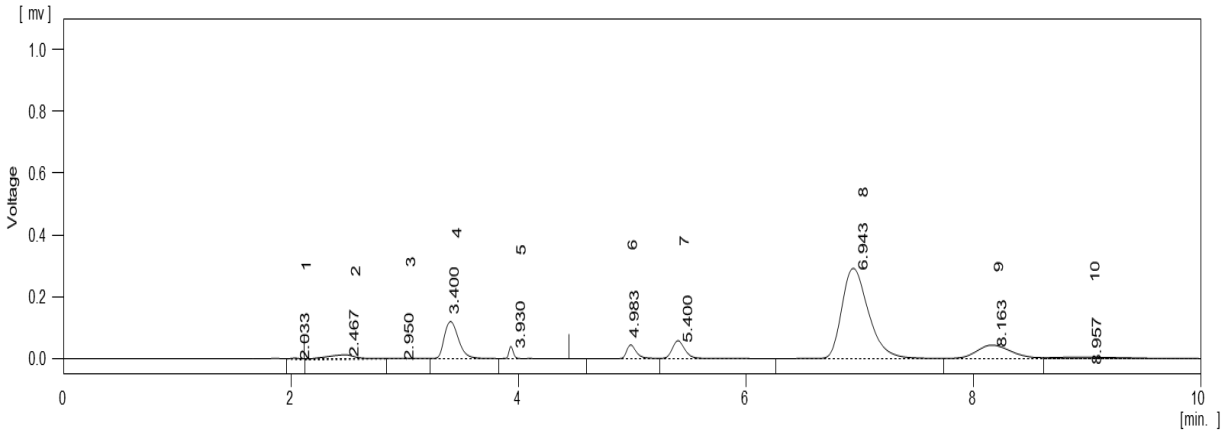

FIG. VII CHROMATOGRAM OF SAMPLE FOR PHOTO DEGRADATION

5. Thermal degradation

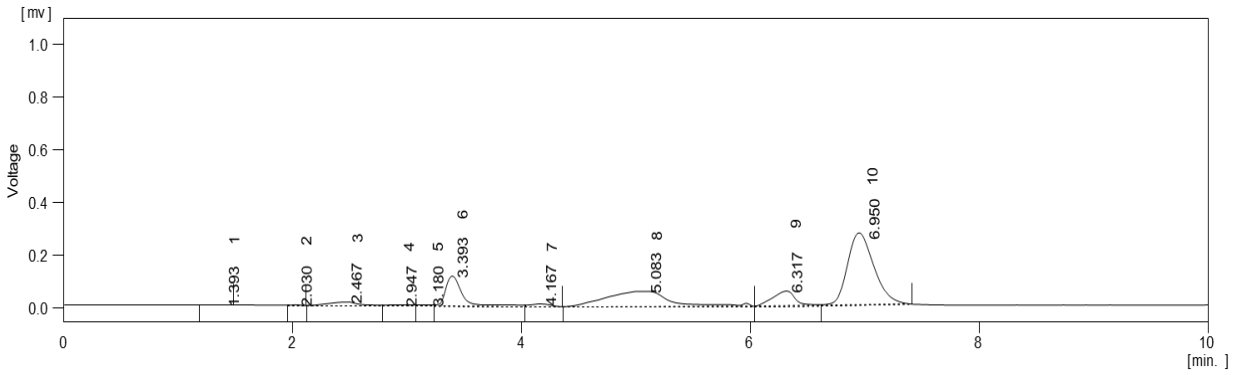

FIG. VIII CHROMATOGRAM OF SAMPLE FOR THERMAL DEGRADATION

TABLE III. DEGRADATION SUMMARY

Stress type	Condition	NEBI	CIL
Acid	Tab_0.1N HCL at RT for 2 hrs	15.02	19.88
Base	Tab_0.1N NaOH at RT for 2hrs	18.22	16.02
H2O2	Tab_3% H2O2 at RT for 2 hrs	16.80	16.25
Photolytic	Tab_Sun light Exposure for 5 hr	18.09	15.84
Thermal	Tab_Thermal at 105°C for 30 min	16.43	17.37

➢ Method Validation

1. Specificity

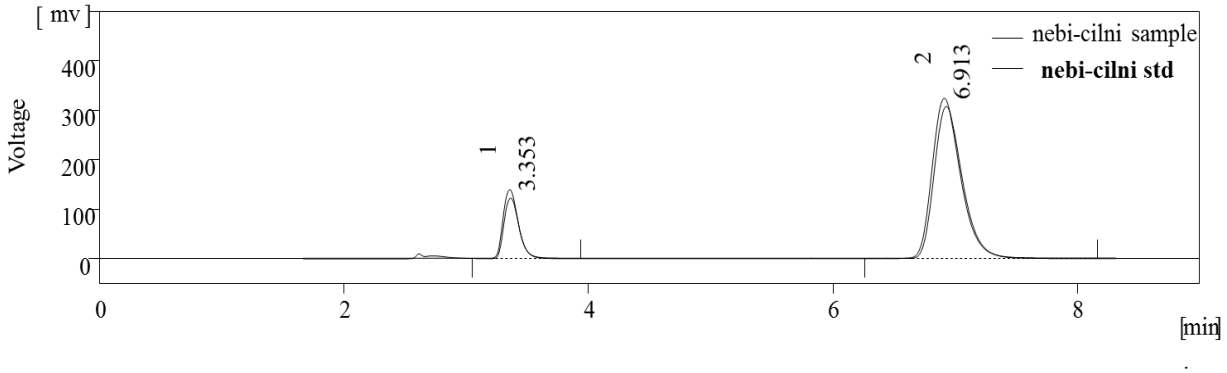

FIG. IX CHROMATOGRAM OF SPECIFICITY

2. Linearity

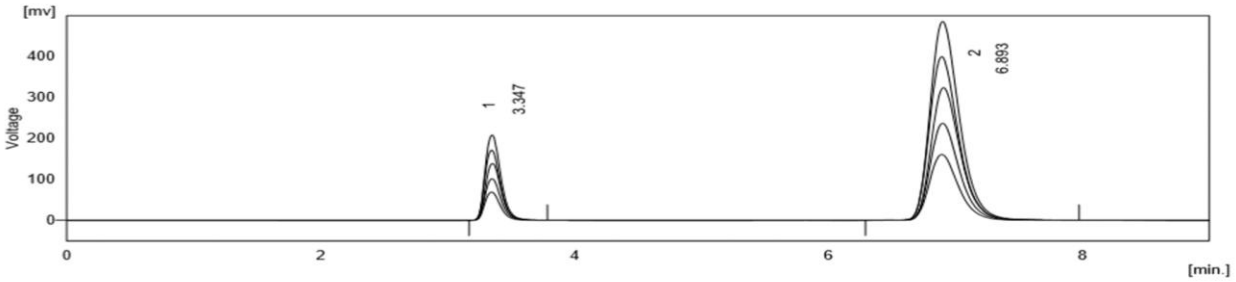

FIG. X OVERLAIN CHROMATOGRAM FOR LINEARITY

TABLE IV. LINEARITY DATA FOR NEBI AND CIL IN HPLC

Conc. (µg/ml)		Mean Area ± SD		% RSD	
NEBI	**CIL**	**NEBI**	**CIL**	**NEBI**	**CIL**
2	4	584.625 ± 0.840	2575.318 ± 0.544	0.142	0.021
4	8	863.128 ± 0.890	3805.611 ± 0.441	0.103	0.011
6	12	1184.572 ± 0.546	5203.436 ± 0.463	0.046	0.008
8	16	1458.703 ± 0.447	6295.142 ± 0.448	0.032	0.007
10	20	1774.796 ± 0.705	780.785± 0.446	0.039	0.005

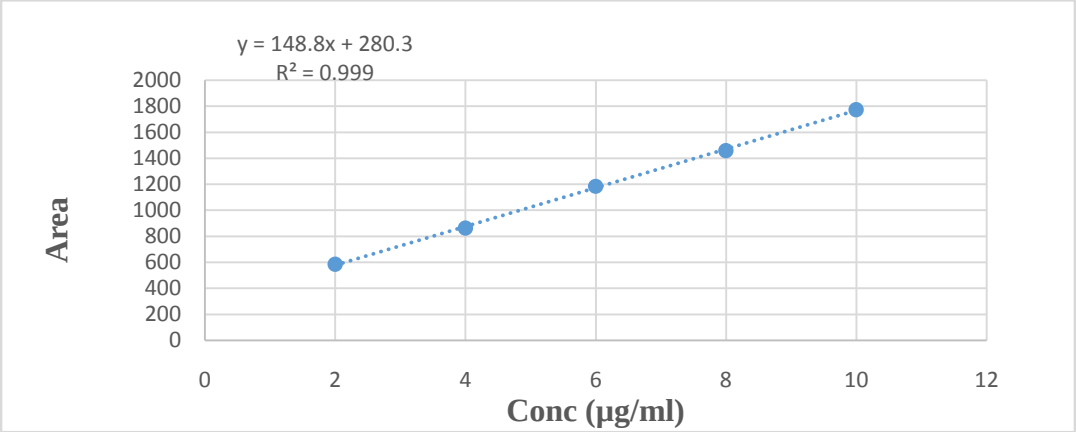

FIG XI CALIBRATION CURVE OF NEBI IN HPLC

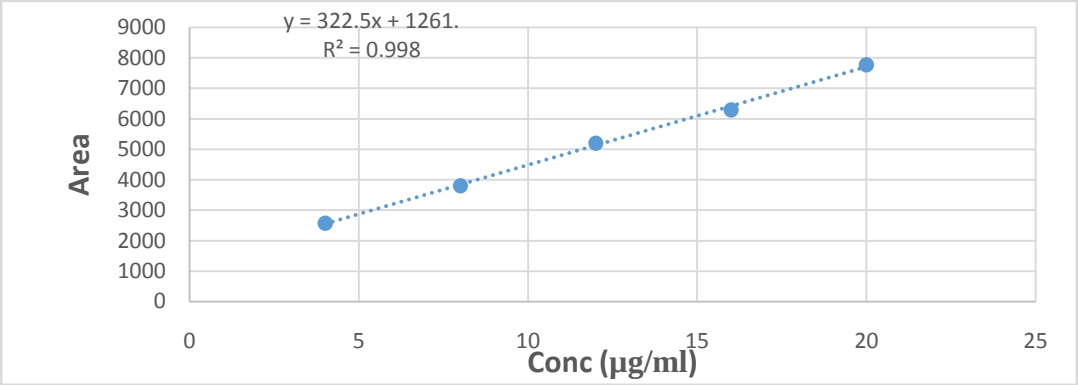

FIG XII CALIBRATION CURVE OF CIL IN HPLC

3. Accuracy

TABLE V. ACCURACY DATA FOR NEBIVOLOL HYDROCHLORIDE (N=3)

AMOUNT OF NEBI (µg/ml)	% OF STD NEBI ADDED	AMOUNT ADDED (µg/ml)	TOTAL SPIKED AMOUNT	AMOUNT RECOVERED (µg/ml)	% RECOVERY (MEAN ± SD)	% RSD
4	80	3.2	7.2	7.284 7.198 7.193	100.04 ± 0.141	0.140
	100	4	8.0	8.084 8.018 8.003	100.07 ± 0.266	0.265
	120	4.8	8.8	8.780 8.808 8.790	99.85 ± 0.4590	0.45

TABLE VI. ACCURACY DATA FOR CILNIDIPINE (N=3)

AMOUNT OF CIL (µg/ml)	% OF STD CIL ADDED	AMOUNT ADDED (µg/ml)	TOTAL SPIKED AMOUNT	AMOUNT RECOVERED (µg/ml)	% RECOVERY (MEAN ± SD)	% RSD
8	80	6.4	14.4	14.478 14.424 14.391	100.16 ± 0.29	0.29
	100	8	16.0	16.078 16.024 16.016	99.82 ± 0.32	0.32
	120	9.6	17.6	17.661 17.620 17.632	100.12 ± 0.180	0.18

4. Assay

TABLE VII % PURITY DATA FOR NEBI AND CIL

Sr. No	Batch no. of Tablet	Actual amount (mg)		Amount obtained (mg)		% Assay of NEBI ± S.D. (n=3)	% Assay of CIL ± S.D. (n=3)	% RSD of NEBI	% RSD of CIL
		NEBI	CIL	NEBI	CIL				
1	GLNT 15002	5	10	4.97 ± 0.012	10.2 ± 0.1	99.98 ± 0.2750	100.52 ± 0.1734	0.27	0.17
2	GLNT 15003	5	10	4.99 ± 0.011	9.98 ± 0.01	99.80 ± 0.2100	99.98 ± 0.1350	0.21	0.13
3	GLNT 15005	5	10	4.98 ± 0.01	10.05 ± 0.1	99.67 ± 0.2374	100.37 ± 0.1293	0.23	0.12

TABLE VIII. SUMMARY OF VALIDATION

Validation parameters		Nebivolol HCl	Cilnidipine
Linearity range (µg/ml)		2-10 µg/ml	4-20 µg/ml
Correlation coefficient(r^2)		0.999	0.998
Recovery Study	80%	100.04	100.16
	100%	100.07	99.82
	120%	99.88	100.12
Repeatability %RSD (n=6)		1.364	0.511
Intraday Precision (%RSD) (n=3)		0.060	0.008
Interday precision (%RSD) (n=3)		0.077	0.012
Limit of detection (µg/ml) (LOD)		0.008	0.0031
Limit of quantification (µg/ml) (LOQ)		0.024	0.0096
Robustness (%RSD)	Flow rate	1.09 – 1.81	0.65 – 0.95
	Mobile phase	1.39 – 1.48	0.86 – 1.02
	pH	1.11 – 1.21	0.85 – 0.94
Analysis of marketed formulation		99.67 – 99.88%	99.98 – 100.52%

DISCUSSION

- A Stability indicating RP-HPLC method have been developed and validated as per ICH guideline for the simultaneous estimation of NEBI and CIL in pharmaceutical dosage form.
- NEBI and CIL solution were subjected to forced degradation by Acidic(0.1 N HCl), Basic(0.1 N NaOH), Oxidation (3% H_2O_2), Photolytic (Sun light) and Thermal (105˚C) condition. The study was done on both standard and sample drug formulation. NEBI shown highest degradation in Basic condition and CIL shown highest degradation in Acidic condition.
- The study revealed that both drugs was degraded up to 15-20%.
- Linearity of the developed method was near to 0.999, Range was found 2-10 µg/ml of NEBI and 4-20 µg/ml of CIL.%RSD was found to be less than 2 for Linearity, Intraday and

Interday precision. LOD and LOQ for NEBI in this method were found 0.008 µg/ml and 0.024 µg/ml respectively. For CIL, they were 0.0031 µg/ml and 0.0096 µg/ml. Assay of marketed formulation were also done by this HPLC and they were found 99.67 – 100.52 % and other system suitability parameters were found in given limit.

ACKNOWLEDGEMENTS

It gives me an immense pleasure to express my gratitude towards my Guide Ms. Jinal N. Tandel, Dr. Samir K Shah, Principal of Sardar Patel College of Pharmacy, encouragement and positive attitude towards work has instilled more confidence in me.

REFERENCES

1. Indian pharmacopoeia; Indian Pharmacopoeia Commission, Government of India ministry of health and family welfare, Controller of India, 2014; Vol III, 2310-2311.
2. "Drug Bank Open Data and Drug Target Data Base" Nebivolol Hydrochloride, http://www.drugbank.ca/drugs/db04861.
3. "ChemSpider Search And Share Chemistry" Nebivolol Hydrochloride http://www.chemspider.com/Search.aspx?q=Nebivolol%20Hydrochloride
4. Walker R andWhittlesea C (2007). Clinical Pharmacy and Therapeutics; 4th Edn; Churchill Livingstone Elsevier Science Ltd, pp 459.
5. Cilnidipine〝 Drug profile " August 2014,http://www.drugs.com/international/cilnidipine.html
6. "ChemSpider Search And Share Chemistry" Cilnidipinehttp://www.chemspider.com/Chemical-Structure.54833.html
7. Meyyanathan S.N. and Birajdar A.S., Simultaneous estimation of Nebivolol hydrochloride and Valsartan and Hydrochlorothiazide in pharmaceutical formulation by UV spectrophotometric methods." *Indian J Pharm Educ*.,2009; Vol.44(2), 156-159.
8. Soni I.J. and Panchal H.J., Development and Validation of Dual Wavelength UV Spectrophotometric Method for simultaneous estimation of Cilnidipine and Olmesartan Medoxomil in Tablet dosage form", *Ind. J. Pharmaceut. Bio. Res.*, 2014; 76-81.
9. Srujani C.H. and Murthy S.N., Novel RP-HPLC Method Development and alidation for the Simultaneous Estimation of Nebivolol Hydrochloride and Hydrochlorothiazide in Bulk and Pharmaceutical Dosage Form." *Res J Pharm Biol Chem Sci.*,2014; Vol. 5(4),1105- 1112.
10. Manzoor A., Manohara Y.N., and RaviM.C., Development and validation of RP-HPLC method for simultaneous estimation of Nebivolol hydrochloride and hydrochlorothiazide in combined tablet dosage form", *Int J ChemTech Res.*, 2012; Vol. 4(1),328- 336.
11. PawarP., GandhiS.V., Deshpande P.B., Vanjari S., and Shelar S.U., Simultaneous RP-HPLC estimation of cilnidipine and Telmisartan in combined tablet dosage form", *www.pelagiaresearchlibrary.com*,2013; Vol.4(2), 6-10.
12. Vyas H.R., Patel S.S., Ladva B.J., Dr.Nayak B.S.,Patel S.J. and Mahida V.M., Development of stability-indicating hplc method for simultaneous estimation of nebivolol hydrochloride and valsartan in tablet dosage form", *World. J. Pharm. Sci.*2015; Vol. 4(6),662-670.
13. ICH guidelines Q2(R1), "Validation of Analytical Procedure: Text and Methodology." International Conference Harmonisation, IFPMA, Geneva, 1994.